Amended claim 1:

1. (Amended) A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being [either] microbubbles bounded by an evanescent gas/liquid interfacial closed surface[, or microballoons bounded by a material envelope], said method comprising the step of forming the microvesicles in the presence of a physiologically acceptable gas, [or gas mixture comprising a physiologically acceptable gas, or filling preformed microvesicles with said gas, or gas mixture], said physiologically acceptable gas being selected from the group consisting of SF_6, ~~SeF_6,~~ CF_4, $CBrF_3$, C_4F_8, $CClF_3$, [CCl_2F_2,], C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, [CBr_2F_2] and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is [in] injected into the bloodstream of a patient.

Amended claim 2:

2. (amended) A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being [either] microbubbles bounded by an evanescent gas/liquid interfacial closed surface[, or microballoons bounded by a material envelope], said method comprising the steps of:

preforming the microvesicles or precursors thereof under an atmosphere of a first gas; and

substantially substituting at least a fraction of said first gas with a second gas which is a physiologically acceptable gas[, or gas mixture comprising a physiologically acceptable gas], said physiologically acceptable gas being selected from the group consisting of SF_6, ~~ScF_6~~, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, $[CCl_2F_2,]$ C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, $[CBr_2F_2]$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

Original claims 3-7 have not been amended

Amended claim 13:

13. (Amended) A method of making a contrast agent for ultrasonic echography which consists of gas-filled microbubbles [microvesicles] suspended in an aqueous liquid carrier phase, the microbubbles [microvesicles] having resistance against collapse resulting from pressure increases effective when the said suspensions are injected into the bloodstream of a patient, said method comprising the step of forming the microbubbles [microvesicles] in the presence of a physiologically acceptable gas [or gas mixture comprising a physiologically acceptable gas, or filling preformed microvesicles with said gas or said gas mixture], said physiologically acceptable gas being selected from the group SF_6, ~~ScF_6~~, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, $[CCl_2F_2,]$ C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$, $[CBr_2F_2]$ and C_4F_{10}, said gas [or at least a gas in said gas mixture] being such that, under standard conditions, the pressure difference ΔP between pressures at which the bubble counts are about 75% and 25% of the original bubble count is at least 25Torr.

Original claim 14 has not been amended

New claim 15 from original claim 1:

15. A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microbubbles bounded by an evanescent gas/liquid interfacial closed surface, said method comprising the step of forming the microvesicles in the presence of a gas mixture comprising a physiologically acceptable gas, said physiologically acceptable gas being selected from the group consisting of SF_6, ~~SeF_6~~, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

New claim 16 from original claim 1:

16. A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microballoons bounded by a material envelope, said method comprising the step of forming the microvesicles in the presence of a physiologically acceptable gas, said physiologically acceptable gas being selected from the group consisting of SF_6, ~~SeF_6~~, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

New claim 17 from original claim 1:

17. A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microballoons bounded by a material envelope, said method comprising the step of forming the microvesicles in the presence of a gas mixture comprising a physiologically acceptable gas, said physiologically acceptable gas being selected from the group consisting of SF_6, ~~SeF_6,~~ CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

New claim 18 from original claim 2:

18. A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microbubbles bounded by an evanescent gas/liquid interfacial closed surface, said method comprising the steps of:

preforming the microvesicles or precursors thereof under an atmosphere of a first gas; and

substantially substituting at least a fraction of said first gas with a second gas which is a gas mixture comprising a physiologically acceptable gas, said physiologically

acceptable gas being selected from the group consisting of SF_6, ~~SeF_6~~, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

New claim 19 from original claim 2:

19. A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microballoons bounded by a material envelope, said method comprising the steps of:

preforming the microvesicles or precursors thereof under an atmosphere of a first gas; and

substantially substituting at least a fraction of said first gas with a second gas which is a physiologically acceptable gas, said physiologically acceptable gas being selected from the group consisting of SF_6, ~~SeF_6~~, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

New claim 20 from original claim 2:

20. A method of making a contrast agent having resistance against collapse from pressure increases when used in ultrasonic echography, said contrast agent consisting of gas-

filled microvesicles suspended in an aqueous liquid carrier phase, the microvesicles being microballoons bounded by a material envelope, said method comprising the steps of

preforming the microvesicles or precursors thereof under an atmosphere of a first gas; and

substantially substituting at least a fraction of said first gas with a second gas which is a gas mixture comprising a physiologically acceptable gas, said physiologically acceptable gas being selected from the group consisting of SF_6, ~~SeF_6~~, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said microvesicles having resistance against collapse resulting, at least in part, from pressure increases effective when a suspension of said gas-filled microvesicles is injected into the bloodstream of a patient.

New claim 21 from original claim 3:

21. The method of claim 18, in which the gas used in the first step allows effective control of the average size and concentration of the microvesicles in the carrier liquid, and the physiologically acceptable gas added in the second step ensures prolonged useful echogenic life of the suspension for in-vivo ultrasonic imaging.

New claim 22 from original claim 3:

22. The method of claims 19 or 20, in which the gas used in the first step allows effective control of the average size and concentration of the microvesicles in the carrier liquid, and the physiologically acceptable gas added in the second step ensures prolonged useful echogenic life of the suspension for in-vivo ultrasonic imaging.

New claim 23 from original claim 4:

23. The method of claim 15, in which the aqueous phase carrying the microbubbles contains dissolved film-forming surfactants in lamellar or laminar form said surfactants stabilizing the microbubbles boundary at the gas/liquid interface.

New claim 24 from original claim 5:

24. The method of claim 23, in which said surfactants comprise one or more phospholipids.

New claim 25 from original claim 6:

25. The method of claim 24, in which at least part of the phospholipids are in the form of liposomes.

New claim 26 from original claim 7:

26. The method of claim 24, in which at least one of the phospholipids is a diacylphosphatidyl compound wherein the acyl group is a C_{16} fatty acid residue or a higher homologue thereof.

New claim 27 from original claim 8:

27. The method of claim 16, in which the microballoon material envelope is made of an organic polymeric membrane.

New claim 28 from original claim 8:

28. The method of claim 17, in which the microballoon material envelope is made of an organic polymeric membrane.

New claim 29 from original claim 9:

29. The method of claims 27 or 28, in which the polymers of the membrane are selected from the group consisting of polylactic or polyglycolic acid and their copolymers, reticulated serum albumin, reticulated haemoglobin, and esters of polyglutamic and polyaspanic acids.

New claim 30 from original claim 10:

30. The method of claims 16 or 17, in which the forming of vesicles with said physiologically acceptable gas is effected by alternately subjecting dry precursors thereof to reduced pressure and restoring the pressure with said gas, and dispersing the precursors in a liquid carrier.

New claim 31 from original claim 11:

31. The method of claims 16 or 17, in which the filling of the microballoons with said physiologically acceptable gas is effected by flushing the suspension with said gas under ambient pressure.

New claim 32 from original claim 13:

32. A method of making a contrast agent for ultrasonic echography which consists of gas-filled microbubbles suspended in an aqueous liquid carrier phase, the microbubbles having resistance against collapse resulting from pressure increases effective when the said suspensions are injected into the bloodstream of a patient, said method comprising the step of forming the microbubbles in the presence of a gas mixture comprising a physiologically acceptable gas, said physiologically acceptable gas being selected from the group SF_6, ~~SeF_6~~, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said gas or at least a gas in said gas mixture being such that, under standard conditions, the pressure difference ΔP between pressures at which the bubble counts are about 75% and 25% of the original bubble count is at least 25Torr.

New claim 33 from original claim 13:

33. A method of making a contrast agent for ultrasonic echography which consists of gas-filled microballoons suspended in an aqueous liquid carrier phase, the microballoons having resistance against collapse resulting from pressure increases effective when the said suspensions are injected into the bloodstream of a patient, said method comprising the step of forming the microballoons in the presence of a physiologically acceptable gas, said physiologically acceptable gas being selected from the group SF_6, ~~SeF_6~~, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said gas or at least a gas in said gas mixture being such that, under standard conditions, the pressure difference ΔP between

pressures at which the bubble counts are about 75% and 25% of the original bubble count is at least 25Torr.

New claim 34 from original claim 13:

34. A method of making a contrast agent for ultrasonic echography which consists of gas-filled microballoons suspended in an aqueous liquid carrier phase, the microballoons having resistance against collapse resulting from pressure increases effective when the said suspensions are injected into the bloodstream of a patient, said method comprising the step of forming the microballoons in the presence of a gas mixture comprising a physiologically acceptable gas, said physiologically acceptable gas being selected from the group SF_6, ~~SeF_6~~, CF_4, $CBrF_3$, C_4F_8, $CClF_3$, C_2F_6, C_2ClF_5, $CBrClF_2$, $C_2Cl_2F_4$ and C_4F_{10}, said gas or at least a gas in said gas mixture being such that, under standard conditions, the pressure difference ΔP between pressures at which the bubble counts are about 75% and 25% of the original bubble count is at least 25Torr.

New claim 35 from original claim 14:

35. An aqueous suspension made according to the method of claim 32, wherein the physiologically acceptable gas is such that, under standard conditions, and at a rate of pressure increase to the suspension of about 100 Torr/min, the pressure difference ΔP between pressures at which the bubble counts are about 75% and 25% of the original bubble count is at least 25Torr.

New claim 36 from original claim 14:

36. An aqueous suspension made according to the method of claims 33 or 34, wherein the physiologically acceptable gas is such that, under standard conditions, and at a rate of pressure increase to the suspension of about 100 Torr/min, the pressure difference ΔP between pressures at which the bubble counts are about 75% and 25% of the original bubble count is at least 25Torr.

New claims 37-42 to specific gases:

37. The method of claims 1 or 15, wherein the physiologically acceptable gas is selected from the group consisting of CF_4, C_2F_6, C_4F_8, or C_4F_{10}.

38. The method of claim 1, wherein the physiologically acceptable gas is CF_4.

39. The method of claim 1, wherein the physiologically acceptable gas is C_2F_6.

40. The method of claim 1, wherein the physiologically acceptable gas is C_4F_8.

41. The method of claim 1, wherein the physiologically acceptable gas is C_4F_{10}.

42. The method of claim 1, wherein the physiologically acceptable gas is SF_6.

New claims 43-48 to specific gases:

43. The method of claims 16 or 17, wherein the physiologically acceptable gas is selected from the group consisting of CF_4, C_2F_6, C_4F_8, or C_4F_{10}.

44. The method of claim 16, wherein the physiologically acceptable gas is CF_4.